AB Enzymes GmbH – Feldbergstrasse 78 , D-64293 Darmstadt

A Petition to Amend the Australia New Zealand Food Standards Code with a Xylanase Enzyme Preparation produced by *Trichoderma reesei*

EXECUTIVE SUMMARY

The present application seeks to amend Standard 1.3.3. - Processing Aids of the Australia New Zealand Food Standards Code (the Code) to approve a xylanase enzyme preparation from *Trichoderma reesei* produced by AB Enzymes GmbH.

Proposed change to Standard 1.3.3 - Processing Aids

The table to clause 17, Permitted enzymes of Microbial Origin, is proposed to be amended to include a genetically modified strain of *Trichoderma reesei* as permitted source for Endo-1,4 (3)-β-xylanase (E.C. 3.2.1.8).

This application is submitted under a general assessment procedure.

Description of Enzyme Preparation

The food enzyme is a biological isolate of variable composition, containing the enzyme protein, as well as organic and inorganic material derived from the microorganism and fermentation process.

The main activity of the food enzyme is endo-1,4-ß-xylanase (IUB 3.2.1.8). The food enzyme catalyses the hydrolysis of xylosidic linkages in an arabinoxylan backbone (and other ß-1,4-linked xylans) resulting in depolymerisation of the arabinoxylan into smaller oligosaccharides.

It uses xylans as substrate. Xylans are constituents of hemicellulose, a structural component of plant cell walls. Arabinoxylans (also known as pentosans) are highly branched xylans that occur in wheat and rye flour. Consequently, the substrate for endo-1, 4-β-xylanase occurs naturally in vegetable based foods and can be found in various plant materials including the cell walls and endosperm of cereals, such as wheat and barley.

Apart from endo-1,4-ß-xylanase, the food enzyme also contains other enzymatic side activities in small amount, which are typical to the production organism *Trichoderma reesei*. Those include ß-glucanase and cellulase. Adverse effects from side activities of the food enzyme are not considered a concern, due to small amounts and the fact that such enzyme activities have been used and approved for decades in food processing.

The enzyme is produced by submerged fermentation of a genetically modified *Trichoderma reesei* with a xylanase gene from *Thermopolyspora flexuosa* (previously named *Nonomuraea flexuosa*).

The production organism is removed during filtration and is not present in the final enzyme preparation.

Use of the Enzyme

The food enzyme object of the dossier is typically used in baking process and processes of other cereal based products (such as pasta and noodles), starch processing, distilling, and brewing.

In principle, the enzymatic conversion of (arabino)xylans with the help of endo-1,4-β-xylanase can be used in the processing of all food raw materials which naturally contain(arabino)xylans.

Food enzyme preparations are used by food manufacturers according to the Quantum Satis principle, which means that food manufacturers will typically fine-tune the enzyme dosage based on a dose range recommended by the enzyme supplier.

Benefits

This dossier is specifically submitted for the use of endo-1,4-β-xylanase in baking processes and other cereal based processes, brewing, grain processing and potable alcohol production. Below, the benefits of the use of industrial endo-1,4-β-xylanase in those processes are described. The beneficial effects are of value to the food chain because they lead to better and/or more consistent product quality. Moreover, the applications lead to more effective production processes, resulting in better production economy and environmental benefits such as the use of less raw materials and the production of less waste.

Baking processes:

Endo-1,4-β-xylanase can be used in the manufacturing of bakery products such as, but not limited to, bread, biscuits, steamed bread, cakes, pancakes, tortillas, wafers and waffles.

Arabinoxylans provide functional properties during bread making due to their ability to interact with gluten, bind water and provide dough viscosity. Limited hydrolysis of the water-unextractable arabinoxylans with the help of endo-1,4-β-xylanase results in solubilized arabinoxylans with lower molecular weights, which improves the functional baking properties of these polysaccharides.

The benefits of the conversion of arabinoxylans with the help of endo-1,4-β-xylanase in baking are:

- Facilitate the handling of the dough (improved extensibility and stability, reduced stickiness leading to reduced losses of dough)
- Improve the dough's structure and behaviour during the baking step

- Ensure a uniform and slightly increased volume and an improved crumb structure of the bakery product, which might otherwise be impaired by processing of the dough
- Reduce batter viscosity, beneficial in the production process for e.g. waffles, pancakes and biscuits

Endo-1,4-β-xylanase can also be used in the processing of other cereal based products such as, but not limited to, pasta, noodles and snacks, where they can improve the dough processability and accelerate the drying step, thereby shortening the process time. Arabinoxylans provide functional properties during pasta, noodle and snack making due to their ability to interact with gluten, bind water and provide dough viscosity. Limited hydrolysis of arabinoxylans with the help of endo-1,4-β-xylanase improves the functional properties of these polysaccharides.

The benefits of the action of endo-1,4-β-xylanase are:

- Facilitate the handling of the dough
- Increase firmness and reduce oil absorption in instant noodles
- Reduce checking (formation of hair line cracks)
- Accelerate the drying step, thereby shortening the process time.

Furthermore, endo-1,4-β-xylanase has been used in baking and other cereal based products for over 25 years.

<u>Brewing and other cereal base beverages:</u>

During beer production, the xylans present in the cell walls of the grain are partly responsible for wort and beer viscosity - which impairs wort (lautering or mash filtration) and beer filtration. The benefits of the conversion of (arabino)xylans with the help of xylanase in brewing are:

- Decreased wort viscosity
- Faster and more predictable lautering or mash filtration
- Faster and better beer filterability

- Improved extraction yield
- Reduced consumption of beer filtration aids

Grain processing

Cereals are highly complex structures causing technical difficulties during processing when milled and when fractionated to starch, gluten and fibres. Enzyme systems that act on the cereal components, including xylans, are used to ensure smooth and efficient processing, facilitate the separation (by opening the grain structure) and ensure high quality of the polysaccharide and gluten fractions.

Grain processing also covers milling and peeling. Insufficiently hydrolysed grain cell wall components reduce the effectiveness of the mechanical treatments such as milling and peeling.

The benefits of the conversion of (arabino)xylans with the help of xylanase in Grain processing are:

- Reduced viscosity of the wheat flour batter, facilitating gluten and starch separation
- Improved gluten and starch purity due to greater extraction yield of the high value fraction and efficient removal of arabinoxylan
- Energy savings due to less use of process water, lower evaporator costs and decreased production time.
- Degradation of cell wall components increasing effectiveness of the mechanical treatments such as milling and peeling.

Xylanase is typically added in grain processing during the initial steps such as conditioning, homogenization and dough preparation. The result of the grain processing is food ingredients such as flour or cereal fractions such as starch, gluten, fiber. Xylanase is not necessarily inactivated during grain processing process, but the resultant food ingredients (separated fractions) are further used in other food processes where the enzyme will be inactivated.

Use of the fractions obtained after grain processing:

Flour is used as a food ingredient in baking process. The starch fraction might be used as a food ingredient in other food applications such as baking and dairy, or for technical applications (e.g. for paper production), and for ethanol production or alternatively as animal feed. Starch might also be processed into glucose, maltose high fructose and other syrups which are themselves used in a number of food products.

The fibre fraction is used in baking as well as for animal feed.

The gluten fraction is mostly used in baking to improve the properties of the flour. Gluten might also be used in other food applications such as meat processing.

Alcohol Production

In Potable alcohol production the high levels of xylans, cellulose, lichenin and beta-D-glucans results in high viscosity due to the water-binding capacity. High viscosity has negative effects on alcohol production because it limits solid concentration in mashing and reduces efficiency in the mixing, separation and filtration processes.

Xylanase is used in distilling industrial applications prior to the liquefaction of highly concentrated mashes. The benefits of the conversion of (arabino)xylans with the help of xylanase in potable alcohol production are:

- Decrease viscosity of grain mashes
- Better processing (solid/liquid separation, resulting in higher solid concentration during mashing; increase fermentable sugars and improve mass transfer during fermentation)
- Reduce fouling in the heat exchangers and distilling equipment
- Increase flexibility in the choice of raw materials and allow to use more grain and less water
- Potential higher alcohol (ethanol) yield as result of better processing, and thereby less use of raw materials.

- Reduce fuel consumption due to better heat transfer

Safety Evaluation

The food enzyme object of the present dossier was subjected to several toxicological studies to confirm its safety for consumers. The mutagenicity studies showed that the food enzyme does not have the potential to damage the genetic material of living organisms, including mammals. The oral toxicity study showed that the food enzyme does not exhibit signs of toxicity, up to doses that are several thousand times higher than those which are consumed via food.

The product complies with the recommended purity specifications (microbiological and chemical requirements) of the FAO/WHO's Joint Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) for food-grade enzymes.

The product is free of production strain and recombinant DNA.

The safety of the xylanase preparation was confirmed or is under consideration by external expert groups, as follows:

- **France**: The enzyme preparation was safety assessed according to the Guidelines for the evaluation of food enzymes. This resulted in the authorisation of the enzyme product by the French authorities.
- **USA**: A GRAS determination was done and notified to the US FDA in December 2015 (GRN000628). In the reply letter from FDA dated September 14th, 2016, the agency has no questions regarding AB Enzymes' determination that the xylanase enzyme reparation is GRAS for its intended use.
- **EFSA/ EU Commission**: a dossier was submitted in 2014 in compliance with Regulation (EC) 1332/2008 and is currently being reviewed by EFSA. In addition, the same enzyme has also been approved in the EU for the use in feed.

Conclusion

Based on the safety evaluation, AB Enzymes GmbH respectfully request the inclusion of *Trichoderma reesei* expressing a xylanase gene from *Thermopolyspora flexuosa* (previously named *Nonomuraea flexuosa*) in the table to clause 17 of standard 1.3.3.; Permitted enzymes of Microbial Enzymes.